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Profiling a possible rapid extinction event in a long-lived species

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ABSTRACT

Infectious disease is a contributing factor to species extinction or endangerment. Extinction is most likely to occur when a pathogen is evolutionarily novel, or when it utilizes an alternate reservoir, or when hosts have small pre-epidemic populations. Here we focus on such a case where a mystery disease almost drove the range restricted, Bellinger River Snapping Turtle (*Myuchelys georgesi*), in north-eastern NSW, Australia, to extinction in less than a month. The disease did not affect other turtle species, and the juvenile population of *M. georgesi* appears unaffected. The cause of the disease remains unknown, but may be a novel virus and whether the species can recover with or without human intervention is currently unknown. Here, we review the biology of *M. georgesi* and explore the epidemiology of the disease. We highlight circumstantial evidence of the potential role of recent environmental change in the susceptibility of *M. georgesi*. We show that long-term regional warming and localised drying reduced both water levels in the river and the number of flooding events that occurred prior to the disease outbreak. We also provide evidence that the food web may have been disrupted, possibly causing malnutrition and reduced immunocompetence of sub-adult and adult turtles. We hypothesize that these factors may have exacerbated the virulence and contagiousness of a novel, but as-yet unidentified pathogen, and must be also mitigated in any future recovery actions. The identity of the pathogen is necessary for managing the recovery of the species, however, understanding the processes that rendered the species susceptible to infection is of equal importance for planning the recovery of the species from the brink of extinction.

1. Introduction

Infectious disease is infrequently listed as a contributing factor to species extinction or endangerment. The IUCN Red List (Baillie et al., 2004) reports that in the past 500 years, 100 plant and 733 animal species are known to have gone extinct. Of these 833 known extinctions, only 31 cases (3.7%) have been attributed, at least in part, to infectious disease. Whereas some forces, such as habitat loss or over-exploitation, are listed as the single causal driver of a species' extinction, in no case is infectious disease listed alone (Baillie et al., 2004). Amphibian pandemics caused by Chytrid fungus (*Batrachochytrium dendrobatidis*) may be an exception, but Chytrid extinction risk may also be worsened with climate change (Pounds et al., 2006). This raises the possibility that infectious disease is less likely than other drivers of species extinction to act in isolation. It is critical that we combine evidence with theory to identify the circumstances under which infectious disease is most likely to serve as an agent of extinction. Extinction is most likely to occur when a pathogen is evolutionarily novel to a susceptible host species, or when it utilizes an alternate reservoir (biotic or abiotic), or when hosts have small pre-epidemic populations (De Castro and Bolker, 2005; Gerber et al., 2005).

Identifying the cause of wildlife diseases is difficult because single factors can rarely be identified as solely responsible. In addition to immune suppression related to elevated stress responses and pollutant exposure, environmental change can impinge directly on wildlife health, and may affect population viability in intricate ways. For example, climate-related shifts in pathogen and host ranges, and pathogen spillover among species brought into contact in novel ways, can increase exposure to new diseases (reviewed in Smith et al., 2009). Similarly, changes in habitat size or quality can lead to reductions in prey populations and increased competition for resources (Ryall and Fahrig, 2006), which in turn can cause malnourishment or starvation and increased susceptibility to disease. Effects may be further complicated if the genetic diversity of the species is low, as low genetic diversity has

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been correlated with reduced fitness and lowered evolutionary potential (Spielman et al., 2004).

In the summer of 2014/2015 in Australia, two closely related species of turtle suffered mass mortality event. An unusual mortality event in Johnstone River Snapping turtles (*Elseya irwini*) in Far North Queensland, Australia, occurred during the summer months of December 2014 and January 2015 (Ariel et al., 2017). Moribund animals appeared lethargic with variable degrees of necrotising dermatitis and while the primary etiology of the disease remains unknown, environmental conditions at the time may have played some part, as the conditions were hot and dry and water levels were extremely low (Ariel et al., 2017). Similarly, on the North Coast of NSW, over 2000 km away from the North Queensland mortality event, over 400 Bellinger River Snapping Turtle (*Myuchelys georgesi*) were also found lethargic with variable degrees of necrotising dermatitis and similarly, the primary etiology of the disease remains unknown (Moloney et al., 2015). Importantly, environmental conditions were hot and dry and water levels were extremely low. *Myuchelys georgesi* only exists in the Bellinger River catchment and the mortality event was also a possible extinction event.

Our study reviews the published literature on *M. georgesi* and explores the epidemiology of the unknown disease to determine whether Bellinger River Snapping turtles were particularly susceptible to viral infection. In lieu of peer-reviewed information regarding the novel pathogen itself, we aimed to identify potential associated risk factors that may have reduced immunocompetence of the species. In particular, we sought to identify trends in environmental factors, including temperature, rainfall, and river level, which may have created poor biophysical conditions for *M. georgesi* survival. We also use stable isotope and body condition analyses to determine whether turtles have experienced nutritional deficits prior to the disease outbreak. Finally, we use population viability modelling to determine the chances of extinction of the species under several management scenarios. Our study is admittedly preliminary, and our ultimate aim is to identify any potential environmental deficits that must be mitigated simultaneously with pathogen management to prevent extinction of *M. georgesi*. We hope that our approach and results may also be useful in preventing similar rapid extinction events in other species.

1.1. The event: epidemiology of the disease (summarized from Moloney et al., 2015)

A severe mortality event in Bellinger River Snapping Turtles (*Myuchelys georgesi*) was investigated on February 18, 2015 by Bellingen Shire Council (BSC), Environment Protection Authority (EPA), and National Parks and Wildlife Service (NPWS) following a report of dead or dying turtles on the side of the Bellinger River from local kayakers. An estimated 432 *M. georgesi* were captured with symptoms of a disease or were found dead. Most were slow moving and apparently blinded by inflammation of the eye and surrounding tissues. No other species appeared affected (e.g., native *Chelodina longicollis*, exotic *Emydura macquarii*).

Turtles were initially treated by veterinarians but because of high rates of mortality and potential biosecurity risk, ill turtles were humanely euthanased (Moloney et al., 2015). The Australian Registry of Wildlife Health at Taronga Conservation Society Australia conducted gross and histological examinations of affected animals collected from the riverbank, and coordinated the diagnostic investigation. Turtles displaying initial signs of disease were emaciated and had swollen eyes (Fig. 1), had slight clear nasal discharge, and some animals had hind limb paresis. Necropsy and histopathology revealed turtles were thin and had numerous additional symptoms associated with bacterial infection, but these infections were assumed to not be the primary cause of mortality (Moloney et al., 2015). A range of infectious pathogens were excluded as the primary pathogen for the disease including Ranavirus, adenovirus, paramyxovirus (ferlavirus), herpesvirus, mycoplasma, chlamydia, and trichomonas (Moloney et al., 2015). No toxins

Fig. 1. External symptoms of the disease in *M. georgesi*. (Source: Photo Credit: Rowan Simon).

or environmental contaminants were detected, but only water quality was investigated, and the potential for *M. georgesi* to bioaccumulate contaminants via diet (e.g., Hopkins et al., 2002) is unconfirmed. Scientists at the Elizabeth MacArthur Agricultural Institute (EMAI) Virology Laboratory have recently (July 2015) detected high levels of a novel virus in tissues of affected turtles (Moloney et al., 2015). However, the identity of the virus, and the data supporting its discovery, have not been published at this point.

After initial discovery, the disease appeared to be propagating upstream at a rate of *ca* 2 km per day Moloney et al., 2015). Surveys began in the upper sections of the River to remove healthy individuals from 8th–12th April 2015. Seventeen healthy individuals of a range of sizes and sexes were removed from the top of their range in the River and relocated to a quarantine facility for holding and breeding. Subsequent surveys not long after the removal of these virus-free or asymptomatic adult turtles revealed dead turtles from the same waterholes.

1.2. Post-mortality developments

From a scientific point of view, very little has emerged about the remaining population and the disease since the release of Moloney et al. (2015). A press release suggested the disease was a “Mystery Virus” in September 2015, based on the Moloney et al. (2015) report (ABC News, 2015). However, data supporting this assertion remain unpublished. Twenty juveniles were collected in limited surveys of the river in November 2015 (Bellingen Courier, 2015) and a survey in March 2016 confirmed that the remaining turtles in the River are predominately juveniles (Bellingen Courier, 2016).

Myuchelys georgesi is restricted to the Bellinger and adjacent Kalang rivers, and its total population size in the larger Bellinger River was previously estimated at only 4500 (Blamires et al., 2005). Subsequent density estimates from surveys in 2007 and 2014 (Spencer et al., 2007; Spencer et al., 2014) suggested a population size half of that value. Regardless, decreasing population sizes and a severely restricted distribution may have combined to make the Bellinger River Snapping Turtle vulnerable to a novel pathogen, particularly if populations were stressed or malnourished prior to infection.

2. Material and methods

We combine a classical ecological theory approach with forensic analyses by 1) summarizing all current literature on *M. georgesi*, including both scientific and public media reports about the disease 2) drawing from historical data on the species to determine potential long-term changes in population dynamics and individual health of turtles, and 3) reviewing long-term environmental data on temperature, rainfall, and river levels.

Historical data on the species stems from a range of studies that have occurred inconsistently since 2000. The last systematic survey was

conducted in 2007 (Georges et al., 2011; Spencer et al., 2014). Body size measurements were collected from surveys of turtles in 2007 (Georges et al., 2011; Spencer et al., 2014). The same measurements were obtained from dead or dying turtles collected from the River in 2015. In March 2016 (12–13 months after the disease outbreak), the River was surveyed using similar methods to the 2007 survey (snorkelling and traps- see Spencer et al., 2014). We compared the size structure of the historical population and the affected population by examining histograms (percentage of animals) of all turtles captured in 2007, 2015 and 2016 (Kolmogorov-Smirnov test). The emergency response team who collected turtle carcasses in 2015 were not focused on identifying marked individuals from 2007, but once alerted, photos of dead turtles were taken and we were able to identify 18 marked individuals. From these individuals, we examined individual growth (change in body size) between 2007 and 2015. As stated in the introduction, in April 2015, 17 asymptomatic turtles (10 male and 7 female) *M. georgesi* were removed from the river and housed in pairs or threes at Western Sydney University. In captivity, turtles were maintained in 2000 L ponds at 21 °C on a diet of fish, plants (*Vallisneria* spp.), and commercial turtle food mixes (FishFuel co). Turtles were fed a combination of dietary items three times a week and remaining food was removed from ponds within 12 h of offering. Health checks and weighing were performed fortnightly. Body size measurements (plastron length and carapace length) were collected every six months. Body condition scores were generated by the following formula: mass (g) divided by the cube of midline plastron length (mm), and multiplied by 10,000 (Chessman pers. com.). We compared body condition scores of all *Myuchelys georgesi* at the time of collection, six months later in captivity, and of *Emydura macquarii* captured in the same water holes (in April 2015). We also examined the proportion of insects consumed in the diet of *M. georgesi* and related species by reviewing data presented in Allanson and Georges (1999) and Spencer et al. (2014).

We collected claw clippings from the toes of the left hind leg of turtles captured during the 2016 surveys. Similarly, claw samples were taken from frozen turtles that were affected by the disease in 2015. Claws should reflect the isotopic composition of the prey a turtle has eaten over the past ~12 months (Bearhop et al., 2004; Bearhop et al., 2003). Isotopic ratios of carbon and nitrogen in consumer tissues reflect assimilated diets and can be used to trace nutrients through trophic webs (Bearhop et al., 2004). Ratios of carbon isotopes (ratio of ^{13}C to ^{12}C relative to that of a standard; $\delta^{13}C$) are used to trace sources of primary production underlying food webs, because plants with different photosynthetic pathways fractionate carbon isotopes differently (Layman et al., 2011; Layman et al., 2007). Ratios of nitrogen isotopes (ratio of ^{15}N to ^{14}N relative to that of a standard; $\delta^{15}N$) are useful for identifying relative trophic positions (Layman et al., 2011; Gannes et al., 1997). Isotopically light nitrogen is excreted via ammonia, urea or uric acid, while the heavy isotope of nitrogen is stored in the tissues of organisms (Gannes et al., 1997). As a result, consumers have a higher $\delta^{15}N$ signature than their prey, meaning that organisms higher in the food web are enriched with heavy nitrogen in comparison to those lower in the food web (DeNiro and Epstein, 1981). A similar (but weaker) trend is also seen in $\delta^{13}C$ signatures, which are enriched with trophic positions (DeNiro and Epstein, 1978). Sample preparation for stable isotope analysis followed standard methods (Revesz and Qi, 2006). We washed turtle claws in deionized water using a vortex (Van Dyke et al., 2013). Samples were then freeze dried at −40° to asymptotic mass using an Edwards Modulyo Freeze Dryer (Hobson et al., 1997). Dried prey items were homogenized using a Qiagen TissueLyser II and stored samples in desiccator until isotopic analysis (Hobson et al., 1997). Samples were weighed into tin capsules, with c. 1 mg of homogenate (Revesz and Qi, 2006). Packaged samples were placed in 96-well microplates prior to analysis.

Using a Thermo Scientific Delta V Advantage isotope ratio mass spectrometer coupled to a ConfloV and FlashHT at the Centre for Carbon, Water and Food of the University of Sydney, $\delta^{13}C$ and $\delta^{15}N$ of the samples was determined. Samples sealed in tin capsules were loaded into an autosampler, which individually dropped them into a helium flushed oxidation reactor at 1000 °C with an oxygen injection at sample drop resulting in the combustion of the samples. After passage through the oxidation reactor, the combustion gases (at this stage CO_2, NO_x and H_2O) were carried by helium through a reduction reactor converting NOx to nitrogen gas, and subsequently passed through a drying agent to remove H_2O. Nitrogen and carbon dioxide gases were separated by a gas chromatograph and transported into the isotope ratio mass spectrometer, which measured the mass to charge ratio of the different isotopologues ($^{12}C^{16}O^{16}O$ and $^{13}C^{16}O^{16}O$, $^{14}N^{14}N$ and $^{14}N^{15}N$) of the sample combustion gases.

Environmental data on river height (water course levels) and air temperature/rainfall were downloaded from NSW Office of water (http://realtimedata.water.nsw.gov.au/) and Bureau of Meteorology (http://bom.gov.au) respectively. Historical water course levels from the Bellinger River at Thora (lat. −30.4259, lon. 152.7809) and the Manning River at Killawarra (lat: −31.9175, lon: 152.3117) were analysed (daily) from 1983 to 2015. We compared water course levels between the Bellinger and Manning rivers to determine whether any temporal changes in river level were unique or consistent to either system. Long-term rainfall data were collated from the nearest data collection station, at Thumb Creek (lat: −30.68, lon: 152.61) from 1961 to 2014. Cumulative differences from mean daily maximum temperatures over the last five decades and over the last five years were collated from the nearest data collection station, South West Rocks BOM Site number: 059030 (~60 km from Thora).

To examine the effect of the disease on population viability we constructed matrix population projection models (PopTools; Hood 2010). Data on fecundity, adult and juvenile survival, and growth for *M. georgesi* were extracted from Blamires et al. (2005), Spencer and Thompson (2005), and Blamires and Spencer (2013; Fig. 2). We used Vortex 10.0 (Lacy and Pollack, 2014) to assess population viability analysis of the current *Myuchelys georgesi* population, as well as, several headstarting management options. 1000 iterations were run for each scenario. The scenarios and headstarting management options included models where no catastrophic events occurred and a scenario where a catastrophic event occurred once every 100 years.

3. Results

3.1. Changes in size structure

The trend of the disease indicates increasing susceptibility with body size (Fig. 3). In 2007 (D = 0.17 p = 0.97) and 2015 (D = 0.19 p = 0.92), the size structure of the surveyed and sick or dead turtle populations was Fisher-Tippett distributed, with a greater skew towards adult size classes among the sick or dead turtles. The remaining turtles in the River are Log-normal distributed (D = 0.19 p = 0.91) with a skew towards juveniles (Fig. 3).

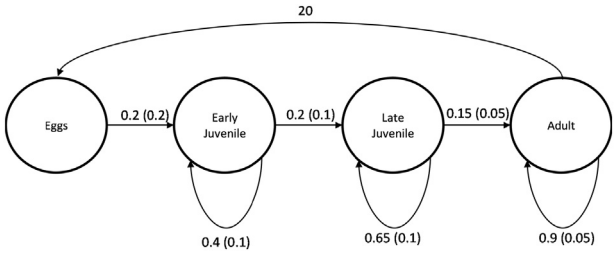

Fig. 2. Mean life cycle parameters developed PVAs on Bellinger River Snapping Turtles. (Source: Data from Blamires et al. (2005), Spencer and Thompson (2005), Blamires and Spencer (2013)).

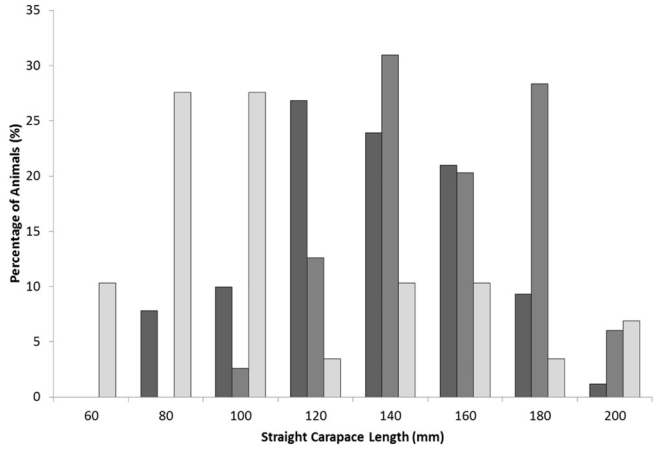

Fig. 3. Population changes in body size. Histograms (percentage of animals) of all turtles captured in 2007 (dark grey) and dead/dying turtles captured 2015 (grey), and the 2016 surveys (light grey).

3.2. Changes in body condition

Based on mark-recapture data, individual turtles also grew by an average of 10.4% ± 2.1% (n = 18) from 2007 to 2015. The body condition of male and female *M. georgesi* brought into captivity increased significantly after six months of captive management and was similar to *Emydura macquarii* captured in the field (Fig. 4a). Relocated turtles did not display any disease symptoms.

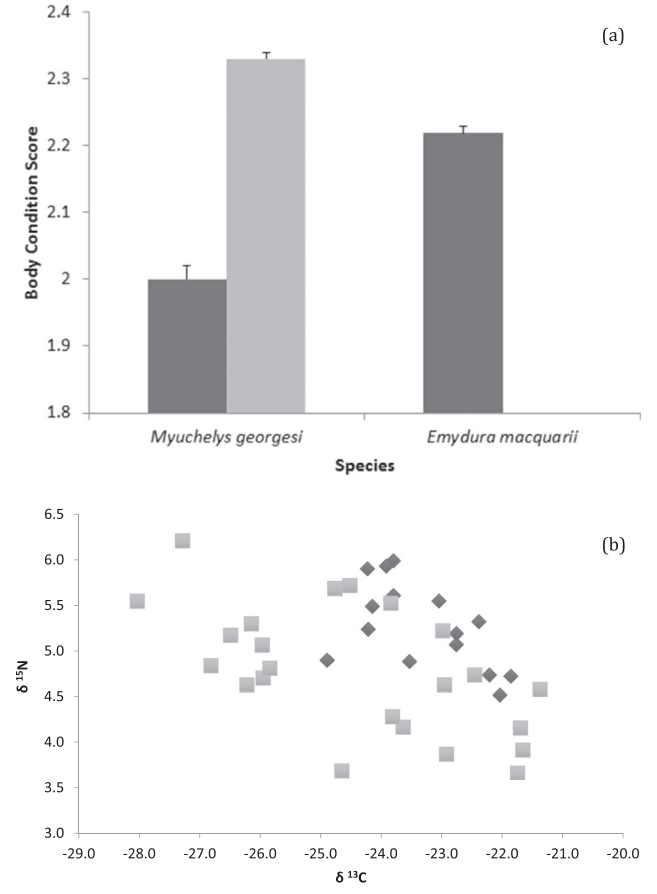

Fig. 4. (a). Body condition score of unaffected *Myuchelys georgesi* and *Emydura macquarii* at capture in April 2015 (dark grey) and after 6 months in captivity (light grey). (b) Isotope profiles of claw samples from the current juvenile (light grey) population and from dead infected (dark grey) turtles in 2015.

3.3. Stable isotope analyses

Juvenile turtles captured in 2016 occupy greater parts of the food web than adults that were affected by the disease, with their $\delta^{15}N$ profile spanning a greater range (Fig. 4b). Similarly, the primary sources of carbon of juveniles are greater than adults with their $\delta^{13}C$ profiles spanning a greater range (Fig. 4b).

3.4. Environmental conditions

Water course levels from 2012 to 2015 at Thora were well below historical averages (Fig. 5a), and dropped 30% below average in 2014. Turtle deaths were recorded less than a week after a minor-moderate flood in February 2015 (Fig. 5b). Over the 32 years of record-keeping, average water course height at Thora was *ca* 2 m, however from Spring 2011–April 2015, water course levels averaged *ca* 1.5 m (Fig. 5b). During this period, only one moderate-major flood occurred in the River until the flood prior to the disease outbreak in February 2015. Low river levels were not directly related to rainfall, because only the last half of 2013 and 2014 experienced significantly reduced rainfall (Fig. 6).

Significant warming has occurred in the region since 1965. Cumulative differences from mean daily maximum temperatures indicated that the degree of warming in the region has been 8–9 times greater in the last decade compared to 1965–1974 (Fig. 7a). Looking specifically at the last five years, the increase of heating (degree-days) in the region in 2014 was almost twice that of 2010 (Fig. 7b).

3.5. Risk of extinction

The probability of extinction after one catastrophic even is 31%, however it increases to 68% if another catastrophic even occurs over the next 200 years. Stochastic population growth (r) declines from 0.04 to −0.02 (Fig. 8). When population numbers are at levels predicted prior to the disease outbreak, the Bellinger River Snapping Turtle appears able to recover from a catastrophic event, however, the recovery is slow and takes over 100 years and the PVA shows that if another catastrophic even occurs within this period, the probability of extinction is 97%.

4. Discussion

The present study reveals how emergent diseases and environmental change might combine to cause rapid and substantial declines of species with restricted ranges. Such findings may not represent an isolated case, as future climatic scenarios should increase the severity of droughts and elevated temperatures (Vörösmarty et al., 2000; Hughes, 2003) which could lead to malnutrition- or stress-related diseases in susceptible species, including rare and endemic species. A novel pathogen has likely killed the adult population of *M. georgesi* (Moloney et al., 2015), but largely left juveniles unaffected in the Bellinger River, and no other species have apparently been affected. The recovery of *M. georgesi* currently relies primarily on a handful of adults rescued to captivity before they became victims of the disease, and a wild population of juveniles. The likelihood of extinction is very high.

Can a pathogen be so contagious and host-specific to travel upstream at *ca* 2 km per day and affect only the adult population of a single species? Viruses most commonly detected in reptiles include herpesviruses (especially in chelonians), adenoviruses (especially in lizards and snakes), reoviruses, (especially in lizards and snakes), paramyxoviruses (especially in snakes), picornaviruses (in tortoises), iridoviruses and ranaviruses (detected predominantly in chelonians) and invertebrate iridoviruses detected in lizards (Marschang, 2011). One possible explanation is that an additional host species was

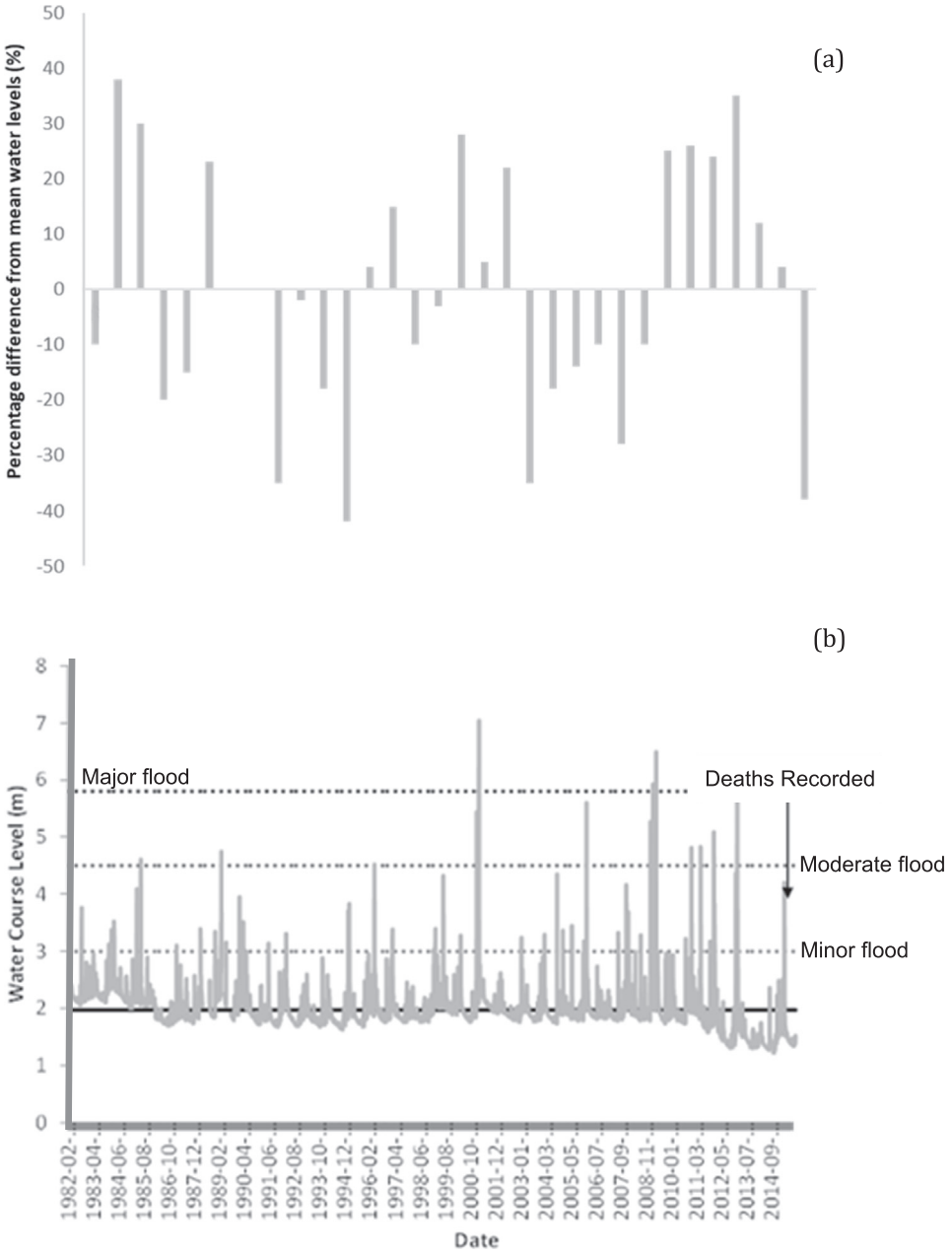

Fig. 5. (a). Annual changes from the mean in water course levels from 1983 to 2014 in the Bellinger River at Thora (lat. −30.42, lon. 152.78) and (b) Monthly averages of water course levels at Thora from 1982 to 2015. Solid black line is average levels over the same time period. Dotted lines represent minor (3.0 m), moderate (4.5 m) and major (5.8 m) flood levels in the River. Deaths we first observed just after a minor-moderate flood in February 2015. Thora (lat. −30.42, lon. 152.78).

travelling upstream and was consumed by only adult *M. georgesi* (and not juveniles). Intraspecific differences in diet are generally related to size. In *M. georgesi*, juveniles consume very little plant material, ephemeropteran larvae, or odonate nymphs compared to adult *M. georgesi* (Allanson and Georges, 1999). However, we show that juvenile turtles consume a broad range of foods, which includes overlap with the diets of adult turtles (Fig. 4b). Prey like live fish, a prime candidate for rapid upstream transmission, are not common food items because short-necked turtles are rarely able to capture them and primarily consume them as carrion (Chessman, 1984; Spencer et al., 1998). To our knowledge, no dead fish were observed during the February/March 2015 emergency surveys. Ephemeropteran or odonate nymphs are also unlikely to be a source for a disease spread through consumption. The closely related species, *Emydura macquarii*, is exotic to the river, but is a generalist and would consume similar foods (Spencer et al., 2014). However, it is possible that the novel pathogen co-exists with *E. macquarii* within its native range, such that *E. macquarii* is more resistant than *M. georgesi*.

Susceptibility to disease often depends on external factors such as environmental parameters, changes in habitat quality, and the genetic makeup of the populations. Thus, mitigating these factors may be as important to recovering *M. georgesi* as is identifying the pathogenic agent responsible for the disease itself. Furthermore, until data on the pathogen itself are available, mitigating these additional factors may be the only current conservation option. It is relatively unusual for infectious diseases to be the sole cause of endangerment for a species (Smith et al., 2009). Our analysis suggests that the *M. georgesi* population was already in decline and that the unknown pathogen may have proliferated because the *M. georgesi* individuals were potentially already immunocompromised. If a virulent pathogen proliferated through a

Fig. 6. Difference to mean in annual rainfall totals at Thumb Creek (Figtree-Lat: −30.68 Lon: 152.61) from 1971 to 2014.

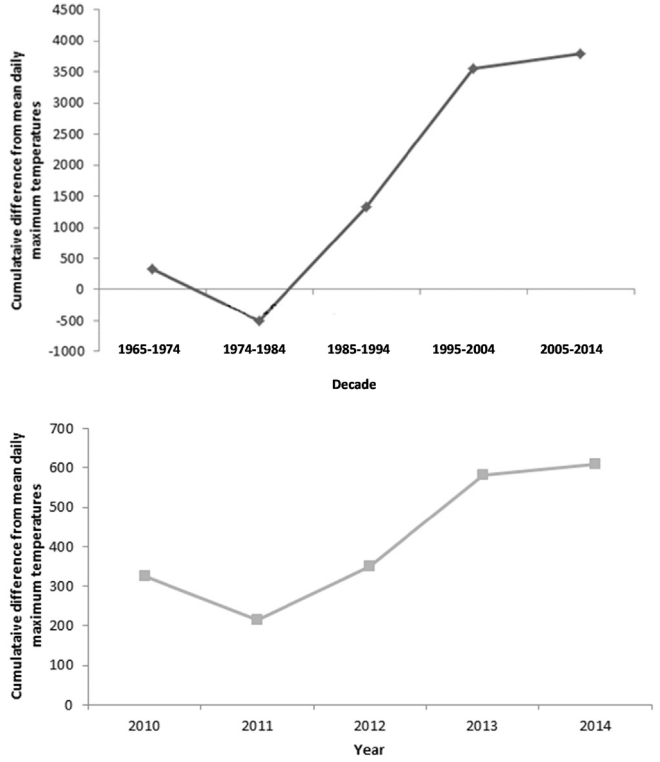

Fig. 7. (a). Cumulative differences from mean daily maximum temperatures over the last five decades and (b) Cumulative differences from mean daily maximum temperatures over the last five years- South West Rocks BOM Site number: 059030 (~60 km from Thora).

healthy population, then the population may have been decimated regardless of cohort or sex, especially if the population exhibited low levels of genetic diversity. In *M. georgesi*, however, the juvenile population escaped the effects of the disease, with turtles less than 100 mm plastron length (probably aged 3–5 y) surviving the outbreak.

The novel virus hypothesis/low genetic diversity argument may help explain the difference in mortality between *M. georgesi* and the introduced *Emydura macquarii* (Georges et al., 2011), but it does not explain the apparent difference in mortality between adults and juvenile *M. georgesi*. If anything, juveniles from the same cohort are likely to have lower genetic diversity than the adult population, because the juveniles are likely to have come from a limited number of nests. *Myuchelys georgesi* can produce more than 20 eggs per nest (Cann, 1998) and if a nest survives, it is likely that most of the hatchlings from the same nest would emerge. Similarly, all of the juvenile turtles captured after the mortality event are from parents that probably did not survive the disease.

Myuchelys georgesi adults may have been malnourished prior to the mortality event. Turtles were described as "emaciated" (Moloney et al., 2015) and our data confirm that turtles rescued for the insurance population had low body condition scores when they were first captured (Fig. 4a). After 6 months in captivity, their body condition scores increased by 18%, similar to wild *E. macquarii* captured at the same time in the River. The virus itself is unlikely to have caused rapid weight loss in *M. georgesi* during the three-week disease event. Turtles, as ectotherms, have low metabolic rates and should catabolize body reserves very slowly (McCue et al., 2012). Indeed, reptiles are well-adapted for surviving long periods without food, so otherwise healthy animals should not become emaciated unless they have undergone long periods without food (McCue, 2010).

Several dietary studies on *Myuchelys georgesi* indicate that they are dietary specialists relative to other short-neck turtles (Spencer et al., 2014). Their diet consists predominantly of insect larvae and terrestrial food that falls on the water surface, like berries, figs, and insects (Fig. 9). We show that ontogenetic differences in diet occur between adult and juvenile turtles, which could be associated with their differences in susceptibility to the virus (Bouchard and Bjorndal, 2006). With a broader diet and lower energetic demands, juvenile *M. georgesi* may not have been immunocompromised, similar to *E. macquarii* which is a dietary generalist (Spencer et al., 2014). In contrast, our data provide circumstantial evidence that the adults may have become emaciated and immunocompromised due to a lack of preferred prey. Benthic invertebrate communities are often used as indicators of aquatic ecosystem health because many species are sensitive to pollution and sudden changes in their environment. Measuring EPT ratios (Ephemoptera (mayflies), Plectoptera (stoneflies), and Trichoptera (caddisflies)) are common indices for measuring aquatic environmental health because they are pollution sensitive taxa and also are major dietary items of *M. georgesi* (Spencer et al., 2014).

The Bellinger River has experienced considerable changes in flow over the past 5 years, although we caution that river level gauges can be affected by changes in river geomorphology. However, River conditions had been different in the lead-up to the event with extremely low water levels prior to Christmas 2014, a severe heat episode in early December 2014 and reported elevated water temperatures (Moloney et al., 2015). The 18 months prior to the disease outbreak were the driest recorded in the upper part of the River and rainfall totals throughout the region were also well below average. Whether these climatic changes are

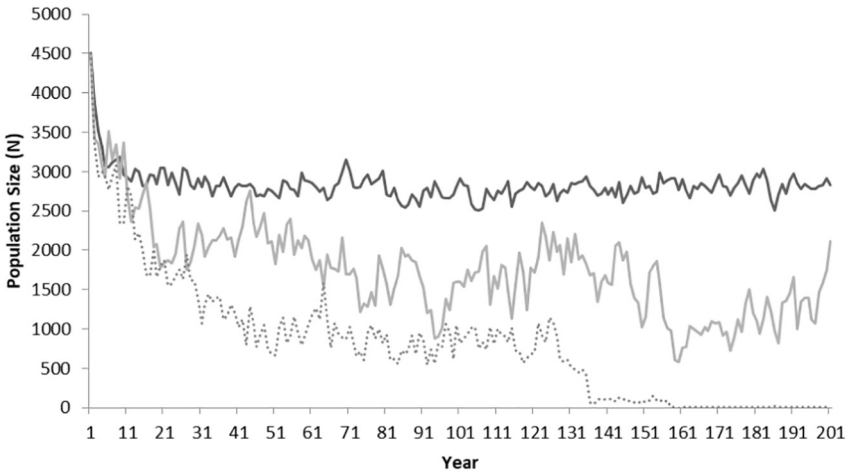

Fig. 8. Simulation using a stable stage distribution of *M. georgesi* based on population size estimated by Blamires et al. (2005). Dark line represents a scenario where no random annual catastrophes occurs once over the time period, light grey line is where a catastrophe occurs once over the time period and the dotted line is where random annual catastrophes occurs twice over the time period (survival rate of all stages reduce to 5% of original values).

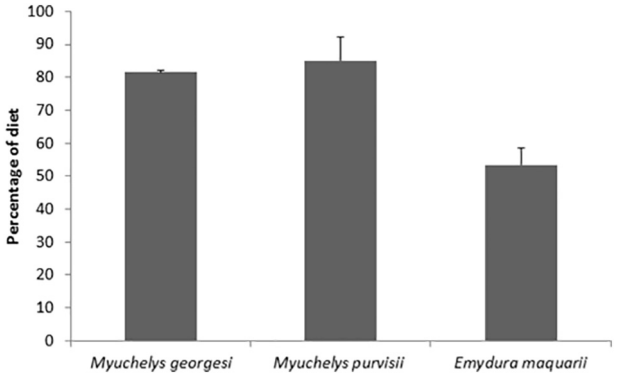

Fig. 9. Proportion of diet that consists of aquatic insects of three short necked species that inhabit clear East Coast River or Lake systems in Australia.
(Source: Data obtained from Spencer et al. (2014)).

responsible for changes in food abundance, leading to potential malnourishment of *M. georgesi*, remains to be tested. Based on our analysis, we hypothesize that susceptibility to the unknown virus in *M. georgesi* may have increased because turtles were immunocompromised because of the direct and indirect effects of climatic change, but this hypothesis requires mechanistic evaluation. Our study may add to the growing body of literature reporting that climate change is having a detrimental effect on organisms. Amphibian and reptilian populations have declined in the lowland forests in Costa Rica in part through the effect of climate change on the humid leaf litter microhabitat of the forest floor (Whitfield et al., 2007). Weather conditions also significantly affect the microclimates for nests and burrows. For example, in sea turtles, elevated temperatures may lead to altered sex ratios or loss of nesting beaches secondary to sea level rises. Temperatures outside the range of those that turtles can tolerate result in the death of the developing sea turtle embryos (Morreale et al., 1982). Accumulation of extreme temperatures experienced by turtle embryos greatly increases the odds that offspring exhibit shell abnormalities that could reduce fitness (Telemeco et al., 2013).

Warming of upper layers in deeper water holes of the Bellinger River also slows down oxygen absorption by water. *Myuchelys georgesi* relies on cloacal bursae (Cann, 1998) for significant gas exchange, and "dead zones", or areas of depleted of oxygen, may significantly impact survival. This is particularly important during their winter hibernation (brumation) period, where their metabolism and oxygen demands are low- it allows them to remain underwater for longer and not use up energy reserves returning to the surface to breathe all the time (FitzGibbon and Franklin, 2010). Persistent dead zones can produce toxic algal blooms, foul-smelling water, and result in fish kills (EPA, 2010).

Myuchelys georgesi stands as a useful case study for improving conservation of species impacted by novel pathogens. We provide strong evidence that adult *M. georgesi* were underweight at the time of the disease outbreak. Asymptomatic turtles were thin before the disease and the numbers of animals dying simultaneously suggests the pathogen opportunistically affected an immuncompromised adult population. Our analysis suggests that diseases caused by novel pathogens can be spectacular climaxes to already declining or stressed populations. Determining the causes and role of malnourishment in disease susceptibility, which in *M. georgesi* was also impacted by demographics, is as important for restoration as is simply identifying and managing for the novel virus. Managing for the virus alone via quarantine methods would likely be insufficient for restoring *M. georgesi* populations. Instead, successful restoration also requires identifying and mitigating the underlying causes of malnourishment. Our results thus emphasize the role of "environment" in the epidemiological triad, as applied to wildlife disease ecology (Acevedo-Whitehouse and Duffus, 2009). If susceptibility of the host to the pathogen is mediated by environmental conditions, then disease epidemics in wildlife populations require managing for those environmental conditions to reduce host susceptibility as much as they require managing for the pathogen itself.

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